

## An Apoptosis-Inhibiting Baculovirus Gene with a Zinc Finger-Like Motif

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Received 26 October 1992/Accepted 13 January 1993

*Spodoptera frugiperda* SF-21 cells infected with *Autographa californica* nuclear polyhedrosis virus mutants which lack a functional *p35* gene undergo apoptosis, a type of programmed cell death. To identify *p35*-homologous genes in other baculoviruses, *A. californica* nuclear polyhedrosis virus DNA containing a deletion in *p35* was cotransfected into SF-21 cells along with genomic DNAs from other baculoviruses. One of the viral DNAs which were able to rescue wild-type infection was from *Cydia pomonella* granulosis virus (CpGV). The CpGV gene responsible for the effect was mapped to a 1.6-kb *SalI*-*SstI* subclone of the *SalI* B fragment of CpGV. The sequence of the *SalI*-*SstI* subclone revealed an open reading frame capable of encoding a polypeptide of 31 kDa which was sufficient to rescue wild-type infection; this gene was thus called *iap* (inhibitor of apoptosis). The predicted sequence of the IAP polypeptide exhibited no significant homology to P35 but contained a zinc finger-like motif which is also found in other genes with the potential to regulate apoptosis, including several mammalian proto-oncogenes and two insect genes involved in embryonic development. In the context of the viral genome, both *iap* and *p35* were able to block apoptosis induced by actinomycin D, indicating that these genes act by blocking cellular apoptosis rather than by preventing viral stimulation of apoptosis. Several independent recombinant viruses derived from cotransfections with either the entire CpGV genome or the 1.6-kb subclone were characterized.

The *Autographa californica* nuclear polyhedrosis virus (AcMNPV) *p35* gene has been shown to be required to prevent premature cell death during infection of some, but not all, insect cell lines (6, 17). Normally, replication of AcMNPV in host insect cells results in formation in the nucleus of virus particles which become embedded within polyhedral occlusion bodies beginning at around 24 h post-infection (p.i.) (for a review, see reference 25). Infection with *p35* mutant viruses, such as the annihilator mutant (vAcAnh), however, results in cell death beginning at around 9 to 12 h p.i., and no polyhedra are formed. Several features of the prematurely dying cells, in particular, the way in which the cells disintegrate by a process of blebbing and the cleavage of cellular DNA into a chromatin ladder, indicate that cell death is due to apoptosis, an active process of programmed cell death commonly observed in vertebrates but not previously confirmed in invertebrates (6).

To gain a better understanding of the mechanism of *p35* action, an assay for detection of *p35*-homologous genes in other baculoviruses was developed. The assay consisted of cotransfection of *Spodoptera frugiperda* SF-21 cells, which undergo apoptosis when infected with *p35* mutant viruses, with vAcAnh DNA and a test DNA. If the test DNA contained a gene able to block apoptosis and thus functionally replace *p35*, the resulting recombinant viruses produced polyhedra, which were easily seen with a light microscope. Although the assay was originally designed to identify *p35*-homologous genes, any gene which can block apoptosis in these cells should be able to rescue polyhedron formation.

In preliminary experiments, we found that *Cydia pomonella* granulosis virus (CpGV) was able to complement the mutation in vAcAnh when DNAs from both viruses were cotransfected into SF-21 cells. The CpGV gene responsible

for this effect, inhibitor of apoptosis or *iap*, was located and sequenced. The predicted polypeptide sequence of IAP, which showed no significant homology to the P35 polypeptide from AcMNPV, contained a zinc finger-like motif similar to those found in several human proto-oncogenes and insect embryonic development genes.

### MATERIALS AND METHODS

**Cells and viruses.** *S. frugiperda* (fall armyworm) IPLB-SF-21 cells (SF-21) (37) and *Trichoplusia ni* (cabbage looper) TN-368 cells (18) were maintained in TC100 medium (GIBCO BRL Laboratories, Gaithersburg, Md.) supplemented with 10% fetal bovine serum (Intergen, Purchase, N.Y.) and 0.26% tryptose broth as previously described (25). AcMNPV annihilator mutant vAcAnh (6) was propagated by using the TN-368 cell line and standard procedures (25). CpGV DNA was obtained from a cloned strain (M1) of CpGV-M (10).

**DNA cloning and sequencing.** Cosmid clones of CpGV DNA, which spanned the complete genome, were selected from a library of clones produced by ligation of a partial *SalI* digest of CpGV DNA into cosmid vector pVK102 (20). The *SalI*-B fragment of CpGV DNA was gel purified from a *SalI* digest of M64 cosmid DNA (see Fig. 1b) and cloned into vector pBluescript SK+ (Stratagene, La Jolla, Calif.). Clones (pSB1 to pSB4) of subfragments B1, B2, B3, and B4 (see Fig. 1d) were obtained by digestion of DNA from a *SalI*-B clone with *XbaI*, *EcoRV*, *ApaI*, and *SstI*, respectively; gel purification of the vector-containing fragment; and religation. Clones pSB5 to pSB7 were obtained from a clone with *SalI*-B in the opposite orientation by digestion with *ApaI*, *SstI*, and *PstI*, respectively, and religation of the gel-purified fragment.

Clones containing a series of overlapping deletions in the *SalI*-*SstI* (B6) region of *SalI*-B were generated by using

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exonuclease III and mung bean nuclease. These were sequenced by the dideoxy-chain termination method using Sequenase (United States Biochemical Corp., Cleveland, Ohio). Sequence assembly and analysis was performed by using the Genetics Computer Group package (12).

**Marker rescue and isolation of recombinant viruses.** A 1- $\mu$ g sample of vAcAnh DNA and 1  $\mu$ g of test DNA (CpGV, cosmid, or plasmid DNA) were cotransfected into  $2 \times 10^6$  SF-21 cells by calcium phosphate transfection as previously described (25). After 3 to 4 days, cells were examined by light microscopy for the presence or absence of polyhedra. Occlusion-positive recombinant viruses were isolated from three independent cotransfections with vAcAnh and either CpGV or pSB6 DNA by plaque assay and subjected to three rounds of plaque purification. Plaque purification and propagation of recombinant viruses were done with SF-21 cells.

**Restriction endonuclease analysis and Southern blotting.** Viral DNA was isolated as previously described (25), and approximately 3  $\mu$ g was digested to completion with various restriction endonucleases in accordance with the manufacturer's directions. The digested DNA was electrophoresed for 16 h at 50 V in a 22-cm 0.7% Seakem agarose (FMC BioProducts, Rockland, Maine) gel in the presence of 0.1  $\mu$ g of ethidium bromide per ml. After photography, the gel was blotted to a Zeta-Probe membrane (Bio-Rad Laboratories, Richmond, Calif.) and hybridized with nick-translated pSB6 DNA or gel-purified *Sal*I-*Sst*I insert DNA from pSB6 (29).

**Inhibition of actinomycin D-induced apoptosis.** Actinomycin D (Pharmacia LKB Biotechnology, Piscataway, N.J.) was added (final concentration, 1  $\mu$ g/ml) to the culture media of uninfected SF-21 cells or to cells infected with various viruses (multiplicity of infection, 20 PFU per cell) at 5 h p.i. Virus titers were determined with TN-368 cells. After incubation with actinomycin D for 10 h, apoptosis was assessed by harvesting total cellular DNA and analyzing the DNA by electrophoresis as previously described (6).

**Nucleotide sequence accession number.** The GenBank/EMBL accession number for the CpGV *iap* gene is L05494.

## RESULTS

**Location of the *iap* gene in CpGV.** Transfection of SF-21 cells with a mixture of vAcAnh and CpGV DNAs resulted, after about 3 days, in foci of infected cells containing polyhedra, whereas control transfections with vAcAnh or CpGV DNA alone produced no polyhedra. The region of the CpGV genome containing the gene able to rescue the wild-type AcMNPV phenotype was located by a series of further cotransfections of SF-21 cells with vAcAnh DNA and cosmids or plasmids containing fragments of the CpGV genome.

The six cosmids used (Fig. 1b) spanned the entire CpGV genome, with overlaps of at least 3 kbp, except at the junction between M17 and M69, where there was no overlap. Only M64 was able to rescue wild-type infection. The location of the gene was further narrowed by using a plasmid clone of *Sal*I-B and seven subfragments, B1 to B7 (Fig. 1d) in plasmids pSB1 to pSB7. The 1.6-kbp *Sal*I-*Sst*I fragment (B6), which was still able to rescue, was sequenced (Fig. 2) and revealed the presence of one incomplete and three complete open reading frames (ORFs) with coding capacities of greater than 50 amino acids (Fig. 3a). None of these ORFs showed any significant sequence homology to the *p35* gene of AcMNPV, and therefore, further cotransfections were done with deletion clones containing subfragments of clone pSB6 (Fig. 3b). Only the clone containing the complete

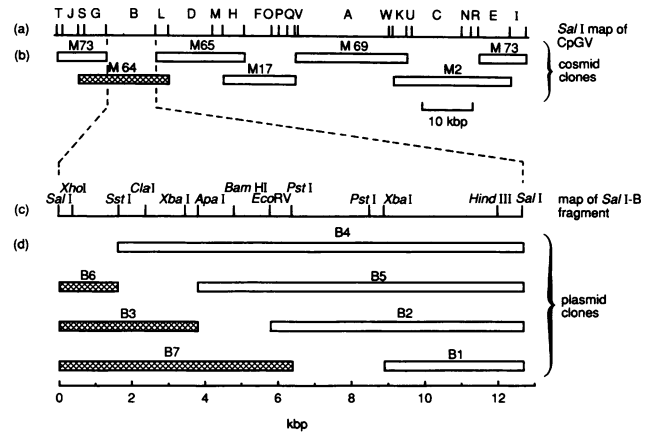


FIG. 1. Locations of cloned CpGV DNA fragments used in marker rescue experiments. (a) *Sal*I restriction map of the complete CpGV genome. The granulin gene is within the *Sal*I T fragment. (b) Bars indicate which *Sal*I fragments were present in each of the six cosmid clones used in marker rescue experiments. The hatched bar shows the cosmid clone which was able to rescue the wild-type phenotype. (c) Restriction map of the *Sal*I B fragment showing selected restriction sites. (d) Bars represent DNA inserts in plasmid clones used in marker rescue experiments. Hatched bars are used in map b.

31-kDa ORF was able to rescue. Other clones containing either of the other two complete ORFs or all of the incomplete ORF present in clone pSB6 did not rescue. This confirmed that it was the 31-kDa ORF which was responsible for inhibition of apoptosis in vAcAnh-infected SF-21 cells, and this gene was consequently referred to as the *iap* gene.

**Sequence analysis of the *iap* gene.** The sequence of the *iap* gene (Fig. 2) exhibited no significant homology with that of the AcMNPV *p35* gene. Furthermore, the predicted IAP polypeptide contained an arrangement of cysteine residues near its carboxy terminus characteristic of a previously

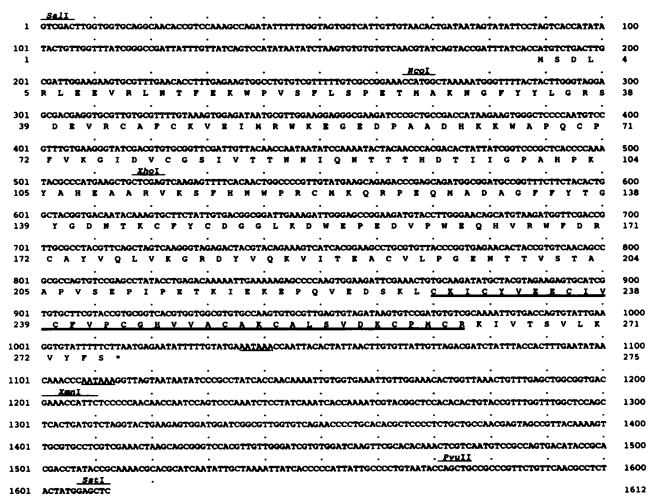


FIG. 2. Nucleotide sequence of the *Sal*I-*Sst*I DNA fragment within CpGV *Sal*I-B (GenBank/EMBL accession no. L05494). The predicted amino acid sequence of the IAP protein is shown below the corresponding codons. Selected restriction sites are indicated, and potential polyadenylation signals are underlined, while the zinc finger-like motif is doubly underlined.

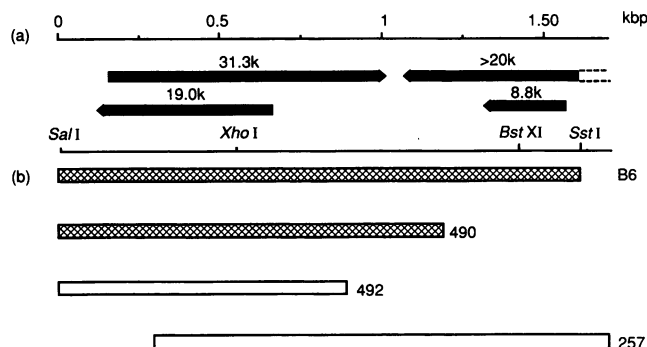


FIG. 3. ORFs in the *SalI*-*SstI* DNA fragment. (a) ORFs with coding potentials of more than 50 amino acids are shown by solid arrows together with the sizes of predicted polypeptides (k, kilodaltons). One of these ORFs starts to the right of the *SstI* site and could code for a polypeptide greater than 20 kDa. Below is a restriction map for selected enzymes. (b) Bars indicate the extent of DNA fragments in four clones used in marker rescue experiments with vAcAnh. Clones B6 and 490 (hatched bars) both contained the intact 31.3-kDa ORF and were able to rescue the virus. The two other complete ORFs and the incomplete ORF were contained within the other two clones (492 and 257, open bars), but neither of these rescued the wild-type phenotype.

described type of zinc finger-like motif, CX<sub>2</sub>CX<sub>10-27</sub>CXHX<sub>2</sub>CX<sub>2</sub>CX<sub>5-16</sub>CPXC (13), where X represents any amino acid. Searches of the most recent available data bases (GenBank release 72, EMBL release 31, SwissProt release 23, and PIR release 33) with this consensus sequence revealed a total of 27 polypeptides from various viral and nonviral sources containing this type of motif. The IAP sequence conformed to this motif very closely (Fig. 4), except that there was an additional amino acid in the central portion, making the sequence CXHX<sub>3</sub>CX<sub>2</sub>C. The other feature of the IAP sequence which was not present in any of the other polypeptides was that the CX<sub>2</sub>C repeat occurred twice instead of just once in the amino-terminal half of the motif, with the more amino-terminal repeat occupying the more conserved position.

Searches of the available data bases revealed two sequences with significant overall homology to *iap*. One of these is located in the AcMNPV *PstI*-I region between 14.7 and 17.9 map units (GenBank/EMBL accession no. M96361; ORF 13 of reference 2a). The CpGV and AcMNPV *iap*-homologous sequences were found to be 45% identical at the nucleotide level and 53% similar and 31% identical at the amino acid level. The level of homology was similar throughout the entire length of the two sequences. Both of the peptides contained the zinc finger-like motif at their carboxy termini. Of the other known polypeptides containing this



FIG. 4. Alignment of IAP, amino acid residues 225 to 266, with a consensus sequence derived from the 27 known zinc finger-like motifs. A single gap was inserted into the consensus sequence to maintain alignment with the IAP sequence. Amino acid positions which are entirely conserved in all of the known motifs are indicated in the consensus sequence, positions occupied by large hydrophobic residues are indicated by dots, and basic, acidic, or amide residues are indicated by an asterisk in the consensus sequence, as described by Wirth et al. (40).

motif, only one has the motif at the carboxy terminus, PAF-1 (27); otherwise, the motifs are found at the amino termini or in the central portions of the polypeptides.

The other sequence found to have significant homology to *iap* also occurs in an insect virus, Chilo iridescent virus (GenBank/EMBL accession no. M81387) (15). When the sequences are optimally aligned, an ORF is found which contains a zinc finger-like motif that is similar to the one found in IAP and has an overall 44% similarity and 25% identity to IAP at the amino acid level.

#### Characterization of annihilator-CpGV recombinant viruses.

Genetic recombination has not been previously observed between members of different baculovirus subgroups. It was therefore of interest to determine the extent of alteration which occurred in the vAcAnh genome during its rescue, especially when the entire CpGV genome was used. Three independent isolates of each type of recombinant (vAcAnh rescued with the entire CpGV genome and vAcAnh rescued with plasmid pSB6) were plaque purified and amplified with SF-21 cells. Preparations of DNAs from the three recombinants made with the CpGV genome (vACP-1, -2, and -3) and the three recombinants made with pSB6 (vASB6-1, -2, and -3) were digested with various restriction endonucleases, and fragment patterns were compared with digested vAcAnh DNA. Comparison of vACP-1, -2, or -3 with vAcAnh failed to reveal any gross alterations, such as loss or gain of any DNA fragments, when the DNAs were digested with *PstI* (Fig. 5A). Similar results were obtained following digestion with *EcoRI*, *HindIII*, *SstI*, *SstII*, or *XhoI* (data not shown). However, some restriction fragments were present in submolar quantities (Fig. 5A). Significant alterations were apparent in the *HindIII*-F, *EcoRI*-I, and *PstI*-D fragments of vASB6-1; the *EcoRI*-A, *HindIII*-D, and *PstI*-G fragments of vASB6-2; and the *EcoRI*-K, *HindIII*-E, and *PstI*-F fragments of vASB6-3 (*PstI* data are shown in Fig. 5A). The other enzymes used for analysis produced altered fragments which either comigrated with other bands or were too large to resolve small size differences consistent with insertion of the plasmid. Given the observed alterations in the restriction fragment patterns of the recombinant viruses, the sites of insertion of pSB6 were localized to between 0 and 3.4 map units for vASB6-1, between 8.65 and 13.4 map units for vASB6-2, and between 25.0 and 29.0 map units for vASB6-3 (25).

Southern blotting confirmed the presence of pSB6 sequences in all six of the recombinant viruses (Fig. 5B). However, a significant difference in signal intensity was consistently observed between the two types of recombinants, with vACP-1, -2, and -3 being at least 10-fold lower in intensity than vASB6-1, -2, and -3 (Fig. 5B), despite the loading of similar amounts of total DNA (Fig. 5A). This difference in intensity was not due solely to the presence of plasmid vector sequences in the hybridization probe used, since similar results were obtained with a gel-purified fragment of pSB6 insert sequences as a probe (Fig. 5C).

The *iap* ORF was intact in all six of the recombinant viruses, further supporting the conclusion that the IAP gene product was responsible for rescue of vAcAnh. Digestion of CpGV DNA with *SalI* and *XmnI*, which cut on either side of the *iap* ORF (positions 1 and 1206 [Fig. 2]), produced a 1.2-kb band which hybridized with the labeled pSB6 *SalI*-*SstI* insert (Fig. 5C). *SalI*-*XmnI* digestion of each of the six recombinant virus DNAs generated the same 1.2-kb band (Fig. 5C). Additional bands also hybridized with the pSB6 insert; most of these additional bands could be attributed to hybridization with the pSB6 insert DNA lying between the

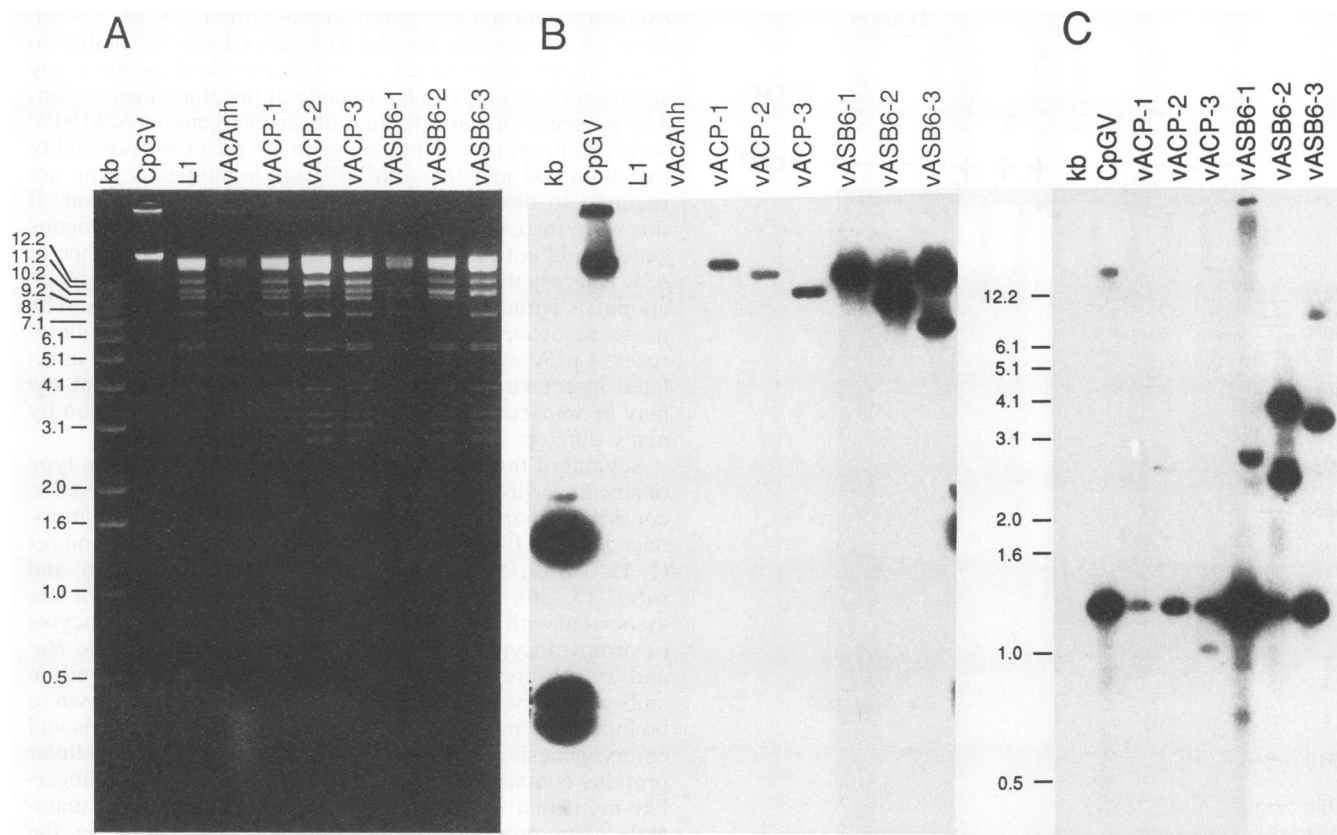


FIG. 5. Analysis of vAcAnh-CpGV recombinant viruses by restriction endonuclease digestion and Southern blotting. (A) *Pst*I-digested DNAs from CpGV; wild-type L1 AcMNPV; vAcAnh; vACP-1, -2, and -3; and vASB6-1, -2, and -3 were separated by agarose gel electrophoresis and visualized by staining with ethidium bromide. Size markers are shown at the left in kilobases. The CpGV DNA sample was not digested to completion in this experiment. (B) The DNAs from the gel in panel A were transferred to a nylon membrane and hybridized with radiolabeled pSB6 DNA. The resulting hybridization pattern was then visualized by autoradiography. Hybridization to certain bands in the size markers was due to the plasmid vector sequences in pSB6 (see panel C). (C) DNAs from CpGV; vACP-1, -2, and -3; and vASB6-1, -2, and -3 were double digested with *Sal*I and *Xmn*I, which cut on either side of the *iap* ORF, and the digested DNAs were analyzed as described for panel B, except that the blot was probed with gel-purified pSB6 insert DNA. A 1.2-kb band would be expected to hybridize with the probe if the *iap* ORF was intact.

*Xmn*I and *Sst*I sites. However, vASB6-2 and -3 exhibited bands which are most readily explained by assuming that these two recombinants contained more than one insertion of pSB6 sequences.

**Abilities of *p35* and *iap* to block actinomycin D-induced apoptosis.** The possibility existed that *p35* and *iap* prevent apoptosis by preventing viral stimulation of apoptosis rather than by directly blocking apoptotic death. To address this question, we tested the abilities of viruses carrying these genes to block apoptosis induced by a different stimulus, namely, the RNA synthesis inhibitor actinomycin D. Treatment of SF-21 cells with actinomycin D induced apoptosis beginning within 2 to 3 hours, resulting in both apoptotic morphology (7) and chromatin fragmentation (Fig. 6). Cells infected with either wild-type AcMNPV (containing *p35*) or vASB6-1 (lacking *p35* but containing CpGV *iap*) did not become apoptotic when treated with actinomycin D at 5 h p.i. or later, whereas cells infected with vAcAnh and treated similarly with actinomycin D became apoptotic more rapidly than when only infected (Fig. 6). Thus, in the context of viral infection, both *p35* and *iap* were able to block apoptosis induced by actinomycin D. None of the viruses was able to block apoptosis if actinomycin D was added at 4 h p.i. or

earlier (7). Presumably, this was due to the time required for synthesis of viral proteins, including P35 or IAP.

## DISCUSSION

The technique of marker rescue has provided a powerful tool for locating and identifying genes responsible for several AcMNPV mutant phenotypes (23), but this is the first time that the method has been used to locate a gene on a different baculovirus. The phenotype of the mutant virus, vAcAnh, had been previously characterized and shown to result in apoptosis in infected SF-21 cells (6). This dramatic alteration of the normal course of infection results in greatly reduced production of budded virus and no polyhedron formation. Rescue of the wild-type phenotype therefore provides a strong selective advantage and an easily observed phenotypic marker; even low levels of recombination, resulting in incorporation of a gene which inhibits apoptosis, can be readily detected, and the resultant recombinant virus predominates on subsequent passage. Although we used this assay to map a gene in a different baculovirus which was able to block apoptosis, the assay may also prove useful for identifying cellular genes involved in blocking apoptosis.

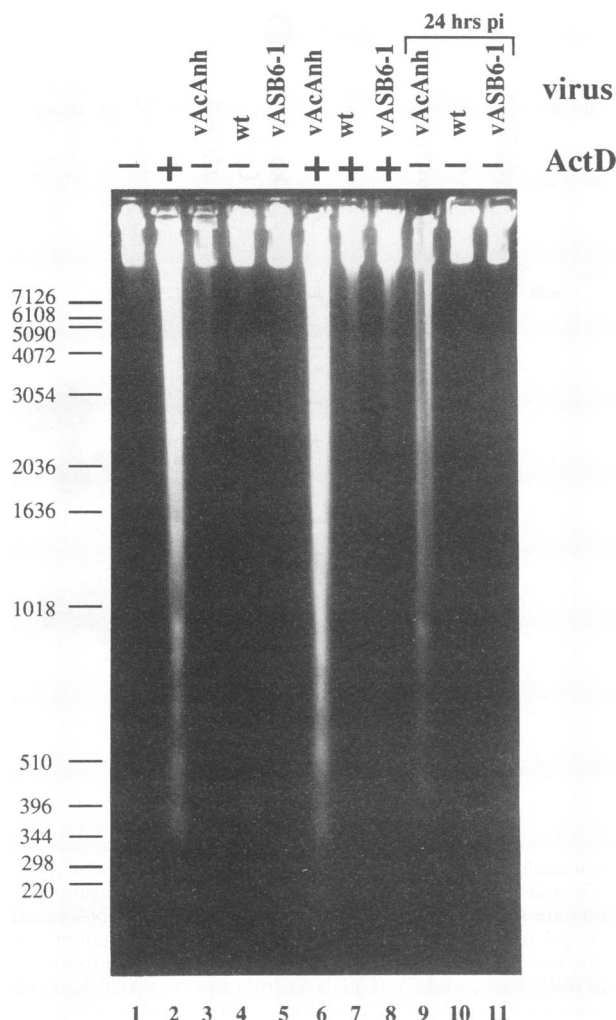


FIG. 6. Abilities of *iap* and *p35* to block actinomycin D (ActD)-induced apoptosis in AcMNPV-infected cells. Total DNA was harvested from SF-21 cells treated as described below, electrophoresed in an agarose gel, and visualized by staining with ethidium bromide. Chromatin fragmentation, which is indicative of apoptosis, is revealed by the presence of an oligonucleosomal ladder consisting of 180-bp multimers. Size markers are indicated at the left in base pairs. Lanes: 1, uninfected SF-21 cells; 2, uninfected cells treated with actinomycin D for 10 h; 3 to 5, cells infected with vAcAnh, wild-type (wt) L1 AcMNPV, or *iap* recombinant virus vASB6-1 and harvested at 15 h p.i.; 6 to 8, cells infected as for lanes 3 to 5, treated with actinomycin D beginning at 5 h p.i., and harvested at 15 h p.i.; 9 to 11, infected cells harvested at 24 h p.i. without addition of actinomycin D.

Southern blotting of AcMNPV DNA with CpGV DNA probes had previously failed to detect any hybridization of CpGV DNA to the *p35* gene region of AcMNPV (9). Nevertheless, it was unexpected that the *iap* gene would show no sequence homology at all to the *p35* gene. The presence of a zinc finger-like motif in the *iap*-encoded polypeptide, together with the lack of overall sequence homology to *p35*, indicates that these two genes are likely to be unrelated in both ancestry and mechanism of action.

This type of zinc finger-like motif has previously been found in three other baculovirus proteins, CG30, PE38, and IEN, from AcMNPV and the homologous proteins in *O.*

*pseudotsugata* nuclear polyhedrosis virus (2, 4, 21, 33–35). None of these other baculovirus genes have the ability to block apoptosis in SF-21 cells (7), nor do they have any significant homology to *iap* outside of the zinc finger region. The presence of a fourth zinc finger-like gene in AcMNPV with significant overall homology to *iap* raises the possibility that both *p35* and the AcMNPV *iap*-homologous gene are required to block apoptosis during AcMNPV infection. If this were the case, however, the AcMNPV *iap*-homologous gene would not be able to block apoptosis in the absence of *p35*, whereas the CpGV *iap* gene apparently is able to block apoptosis without *p35* in vAcAnh-infected SF-21 cells. The presence of *iap* in at least two distantly related baculoviruses, CpGV and AcMNPV, and also possibly in the unrelated insect virus Chilo iridescent virus, suggests that *iap* may be important in blocking apoptosis during infection by many different insect viruses.

Several of the 27 polypeptides known to contain the type of zinc finger-like motif found in IAP may also be involved in control of apoptosis. These include the mammalian proto-oncogenes or oncogenes *PML*, *bmi-1*, *c-cbl*, *mel-18*, and *ret* (1, 11, 16, 19, 31, 32), as well as *Drosophila* genes *Psc* and *su(z)2* (3, 36). In particular, *PML*, *bmi-1*, and *c-cbl* are associated with abnormal proliferation of either lymphocytes or promyelocytes when mutated (1, 11, 16, 19), while *Psc* and *su(z)2* are homologs of *bmi-1* and are involved in embryonic development (3, 36). Since apoptosis is known to be intimately involved in both survival of immune cells and embryogenesis (38, 39), *iap* may belong to a class of cellular proteins containing a conserved and distinctive zinc finger-like motif that regulate apoptosis in both insects and mammals. The presence of the zinc finger-like motif in the *iap*-encoded polypeptide suggests that IAP acts as a transcriptional regulatory factor; it has been suggested (13) that zinc fingers of this type are involved in DNA binding, although this has been demonstrated only for *mel-18* (31).

Characterization of the progeny viruses resulting from the marker rescue experiments was of interest, since examples of recombination between different baculoviruses have only rarely been recorded, consisting of recombination between genotypic variants of the same virus (8, 28, 30). In these cases, recombination apparently occurred at regions of high homology. In the present work, the two baculoviruses involved are only distantly related, and although the gene which was transferred from CpGV shows low but significant homology to the *Pst*I-I region of AcMNPV, the site of insertion apparently varied with each of the recombinants characterized. The three recombinants resulting from cotransfection with pSB6 were plaque purified and gave restriction profiles showing differences from wild-type virus consistent with insertion of a small fragment of DNA at distinct sites between 0 and 30 map units. We wanted to characterize the recombinants resulting from cotransfections with the whole CpGV genome to determine whether large segments of CpGV DNA were incorporated into AcMNPV or whether just the *iap* gene was selected. Despite three rounds of plaque purification, it appeared that the recombinant viruses either had not been cloned or were genetically unstable; the latter possibility seems more likely, given the extensive plaque purification. Each of the viruses contained the intact CpGV *iap* gene, as shown by their normal AcMNPV phenotype and Southern blotting. However, restriction analysis gave profiles more or less similar to that of wild-type AcMNPV, and levels of the *iap* gene present in these viruses were considerably lower than in the pSB6 recombinants, as determined by comparison of signal inten-

sities on Southern blots. The presence of submolar restriction fragments and low levels of *iap* in vACPI-3 suggests that these viruses consist of a mixed population of genotypes.

While treatment of cells with RNA or protein synthesis inhibitors usually blocks apoptotic cell death, the ability of actinomycin D to induce apoptosis has been previously described for a few mammalian cell types (14, 22). Induction of apoptosis by inhibition of RNA synthesis indicates the need for continuous synthesis of a factor required to inhibit apoptosis in these cells. Since treatment with the protein synthesis inhibitor cycloheximide results in only very low levels of apoptosis in SF-21 cells (7), the data indicate the requirement for RNA synthesis to inhibit apoptosis. It should be noted that induction of apoptosis by AcMNPV infection occurs at approximately the same time as a reduction in host RNA and protein synthesis begins, at around 9 to 12 h p.i. (5, 24), suggesting a possible common mechanism of induction of apoptosis by actinomycin D and virus infection.

Although both *iap* and *p35* are able to block apoptosis induced by two very different effectors, viral infection and actinomycin D, it seems likely that they operate in different ways because they share no obvious sequence homology. Different control points along the apoptotic pathway(s) are certainly possible, given the high degree of complexity thought to be involved in apoptotic control (26). Alternatively, it may be that *p35* is only required in some baculoviruses to assist in the function of *iap*. We are currently studying the function of the AcMNPV *iap*-homologous gene in AcMNPV infection to determine whether it has any involvement in prevention of apoptosis. It is also possible that the function of *iap* in CpGV is unrelated to apoptosis blocking, since we have not tested the effect of deleting the *iap* gene on the replication of CpGV in *C. pomonella* cells or insects.

#### ACKNOWLEDGMENTS

N.E.C. and R.J.C. contributed equally to this work.

We thank Ginger Carney for assistance in characterizing the recombinant viruses.

N.E.C. was in receipt of MAFF funding and a fellowship from the OECD Agricultural Project on Biological Resource Management. This research was supported in part by Public Health Service grant AI23719 from the National Institute of Allergy and Infectious Diseases to L.K.M.

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